FURTHER EXPERIMENTS ON THE REGENERATION OF TISSUE COMPOSED OF PARTS OF TWO SPECIES.

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THE experiments that I made a year ago were undertaken in order to find out if regenerated tissue, made up of cells derived from two species, showed any mixing of the specific characters of the two species. For this purpose I grafted the tail of a tadpole of one species of frog upon the posterior end of a tadpole of another species. Later the tail was cut off in such a way (as indicated by the line *b-b* in Fig. 5) that the ectoderm left at the exposed edge belonged in part to one species, in part to the other. When the new tail regenerated there was found to be no mixing of the characters of the ectodermal cells along their line of contact in the new part. The results were unsatisfactory from one point of view, inasmuch as the small piece of ectoderm left after the operation is carried out to the tip of the new tail and increases proportionally less in area than the rest of the new part, so that although it is highly probable that near the tip of the tail new ectodermal cells are being formed by both kinds of ectoderm, still I did not demonstrate that this is actually the case. Moreover, I found that in the later stages the difference in color between the two kinds of ectoderm was less marked than at first, so that the experiment would have been more convincing had the tail been cut off at an earlier stage. This I have done during the present spring, and the results in regard to the ectoderm

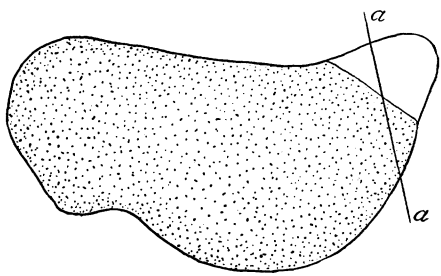


FIG. 1.

confirm in every way those given in my former paper. In the experiments made this spring my main object has been, however, to carry out the experiment in such a way that there would be left at the exposed edge, when the grafted tail was cut off, the internal tissues of two species. In this way I hoped to be able to determine more definitely if, in the newly regenerated part, the tissues mutually influence each other.

The day after the grafting had been performed (*i.e.*, after eighteen to twenty-four hours) the tail was cut off at the region of union of the two components, as shown by the line *a-a* in Fig. 1. In this way there is left at the exposed edge not only the ectoderm of the two species, but the inner tissues also. The regeneration that takes place from the exposed edge will include material derived from both components. Two possibilities presented themselves. First, would the new part be formed of cells intermediate in character between the two species as the result of an interaction of the cells on each other; or, second, would the new material preserve the characteristics of the region from which it arises, or, in other words, one-half of the tail show the characters of one species and the other half of the other species? It is further possible that the new cells might intermingle, and if so the tail might *appear* to be of a hybrid character.

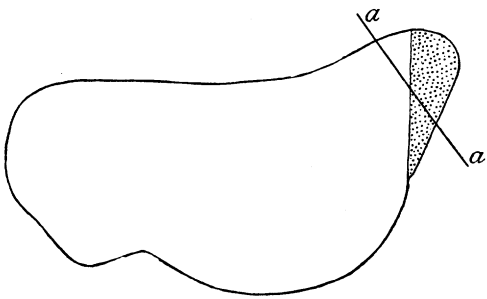
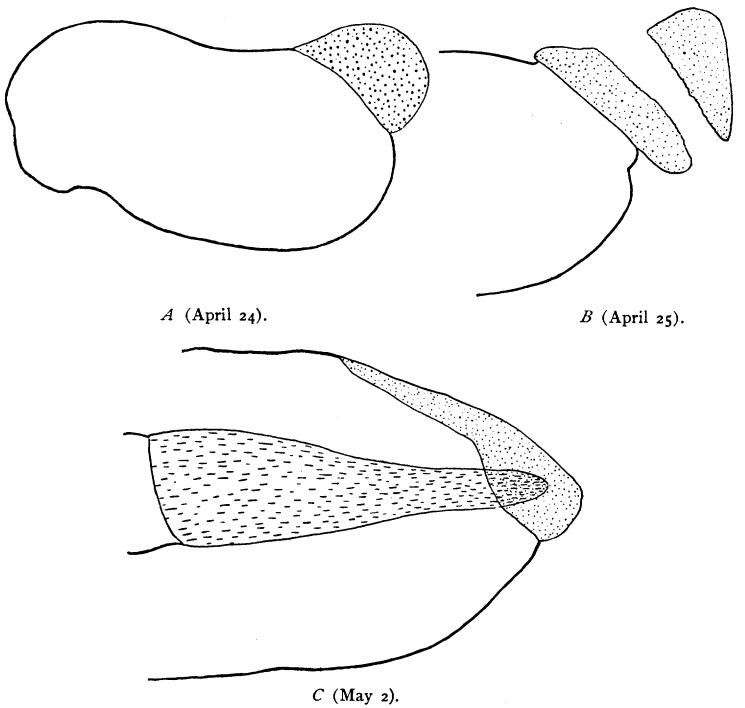


FIG. 2.

Other experiments of minor interest have also been studied. For instance, in several cases the grafted tail was cut off after twenty-four hours very near its line of union to the major component, as shown in Fig. 3, *A*, *B*. The experiment was made in order to see if the major component might not have some influence on the regenerated part from which it is separated by only a narrow band of tissue of the minor component, but no such influence was observed.

I have found a much safer criterion than before for distinguishing the inner tissues of the two species of tadpoles used in these experiments. In my former experiments I used the differences in color of the pigment cells. I find that this cannot be relied upon under all circumstances. But the muscle tissue of the tail of Rana palustris is, especially in the early stages, golden-yellow, while in Rana sylvatica the same cells



A (April 24). *B* (April 25).

C (May 2).

FIG. 3.

are slaty-white. The two kinds of cells can be easily distinguished by means of this color difference. Other experiments have shown also that this difference in color is transmitted to the regenerating tissues in the new tail, so that it can be relied upon in the grafting experiments.

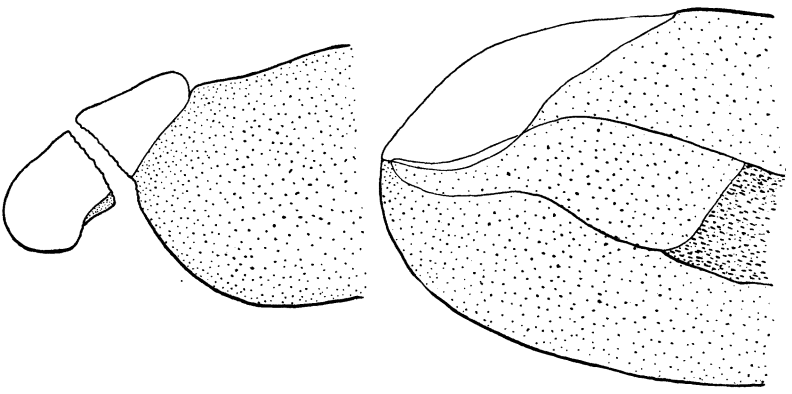
In the first series of experiments the tails were grafted as described in my former paper. After eighteen to twenty-four hours, as a rule, the tail was cut off, as shown by the line *a-a* in Fig. 1. Before grafting it was found more convenient to cut

off the tails obliquely, as shown in the figure — the more anterior end on the dorsal side. Consequently, in order to carry out the second operation of cutting off the tail through the line of union, the cut was made also obliquely, but with the ventral side forward. In a few cases the tails were first cut off with the ventral side further forward (Fig. 2), and the subsequent cutting off was made with the dorsal side forward, as shown by *a-a* in Fig. 2, but the results were practically the same.

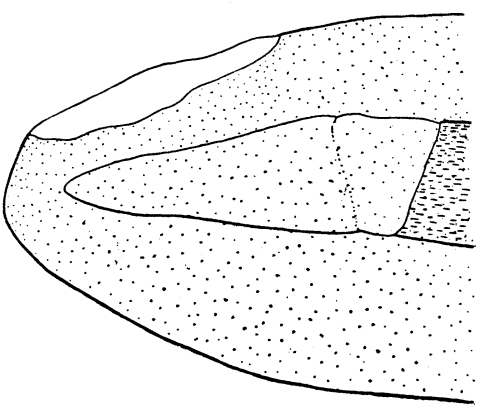
It was found easier to graft the tail of Rana palustris on the posterior end of Rana sylvatica than the reverse. On an average five operations of the former succeeded to one of the latter. The reason for this cannot be given, but it may be due to some difference in the relative sizes of the two components that is more favorable for union in one than in the other way. The result recalls the experiments in cross-fertilization of the eggs in different species, where the crossing can be more easily carried out in one direction than in the other. In this case also the results may be due in some cases to a gross, physical difference, as Pflüger has tried to show for the frog's egg.

In the large majority of cases in which the experiment was carried out as shown in Fig. 1, the core of the new tail seemed to be formed by the minor component, — *i.e.*, if a yellow tail (R. palustris) had been grafted upon a black tadpole (R. sylvatica) and then after twenty-four hours the tail had been cut off obliquely (Fig. 4, *A*), the central part of the new tail would be composed entirely of the yellow tissue derived from the minor component (Fig. 4, *B*, *C*). The small piece of yellow ectoderm is carried out on the new tail and remains near the tip. It covers a larger area than at first, but it increases not nearly so fast as the rest of the new, yellow tissue of the new tail. The distinctive differences in color can only be seen in the core of the tail, *i.e.*, in the cells that form the muscles. On each side of this axial core a broad fin is present containing inside a gelatinous-like substance with scattered cells, but this fin does not show any difference in color in the two species. It is, therefore, probable that in many cases in which the core of the new tail appears to be composed only of tissue from the minor component that the ventral (or dorsal) connective tissue

of the fin is derived from the major component. The differences in the mesodermal pigment cells are at times very striking, and in all such cases the pigment cells are like those in the tissues from which they immediately arise; but while in many



A (April 25). *B* (May 2).



C (May 6).

FIG. 4.

cases they furnish a safe criterion, in others the difference cannot be made out with certainty. On the other hand, the differences in the muscle tissue of the core can always be seen.

The explanation of the result, *viz.*, that the new tail is in most cases (in forty-seven cases out of sixty) like the minor component, is that in nearly all of these operations too large a piece of the grafted tail has been left. It has contained the

notochord and nerve cord and the tissue immediately around them, and from these the new tail has grown out. I did not discover this until most of my material had been used. After this I cut off the grafted tails nearer the line of union, and although regeneration did not take place so well in several cases, still those that regenerated showed more often both parts contributing to the new tail. The same result followed in a number of cases in the previous operations, and we may now examine how regeneration takes place in such cases.

In thirteen cases (out of sixty) there was found evidence of a dual or compound character to the new tail. In all cases observed there was no evidence to show that the duality was the result of the tissues being mixed in character either by commingling of the cells (each cell retaining its specific characters) or by a hybridizing of the cells (due to mutual influence). The duality consisted in each part, regenerating cells like itself, so that definite regions of the new tail were made up of one or of the other kind of tissue. For instance, the new tail might be made up above of the slaty-colored tissue of R. sylvatica and below of the yellow tissue of R. palustris. There is no evidence of a shading of one kind of tissue into the other along the line of meeting, but this point would be very difficult to determine positively. There is further no evidence that the two kinds of tissue are any more commingled at the distal end of the tail than at the base.

In regard to the notochord and nerve cord it is extremely unlikely that the cut would ever pass obliquely through the line of union of the one or of the other, as these structures are very small in cross-section. It is, therefore, probable that in nearly every case the new notochord and the new nerve cord are made up of cells belonging entirely to one component. Furthermore, these two structures lie so near together that it is not probable that the cut would pass between them in such a way that the nerve cord at the exposed edge would belong to one component and the notochord to the other component.

The details of the successful experiments are as follows: On April 14 and 15 nine grafts were made, as shown in Figs. 1 and 2. On April 16 these were cut off, as indicated in Fig. 1, *a-a*,

and Fig. 2, *a-a*, but unfortunately the two lots were not kept separately. On April 29 when again examined new tails had begun to regenerate, and two individuals out of the nine showed that the core of the new tail was compound in character. In both the major component was black and the minor yellow. In one of these the new tail was yellow on the dorsal side and black on the ventral, and in the other the new tail was black on the dorsal side and yellow on the ventral.

In another series the experiment was somewhat different. The grafting took place on April 17. Two days later the ectoderm of the minor component had been carried out further on the tail (Fig. 5), so that at the base of the tail the inner tissues of the minor component were covered by the ectoderm of

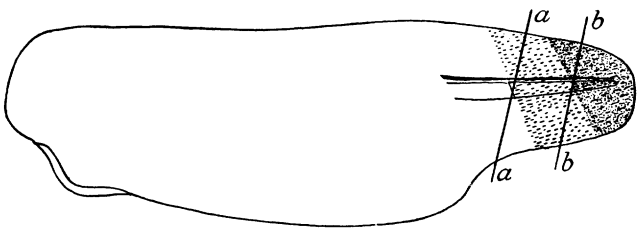


FIG. 5.

the major component. At this time (April 19) the tail was cut off obliquely, as indicated by the line *a-a* in Fig. 4, leaving the inner tissues of both components exposed at the cut surface. On May 19 all three of the tadpoles that had been operated upon showed a compound tail. One of these tadpoles was R. palustris and the other two were R. sylvatica, with grafted tails of the other species, respectively.

In a third series of five individuals, grafted April 19 and cut off April 20, as in Fig. 1, two showed later a compound tail; and in a third I was in doubt whether or not a few of the yellow cells of the major component entered the new tail.

In three later experiments in which the tail had been cut off, so that a smaller piece of the minor component was left attached, a larger number regenerated compound tails.

In one of these experiments the grafting took place on April 27, and the tail was cut off on the following day. One of the three produced a tail composed of both kinds of tissue.

In another experiment grafted April 28 (2.30 P.M.), as shown in Fig. 2, and cut off April 29 (10 A.M.), two individuals formed abnormal tails and a third a compound tail. The tail of this individual is represented in Fig. 6. On the ventral side of the new tail are found the slate-colored cells of the major component, and on the dorsal side the yellow cells of the grafted piece. (It is not possible to show this difference satisfactorily in a simple uncolored drawing, since the principal difference is one of color.) In addition to this difference one can see in the region at which the grafting took place and where the new tissue arises from the old that each component contributes its half to the new tail. Moreover, in all these cases the tadpoles had been carefully observed from day to day (and not only at the intervals recorded in the text) and the gradual formation of the compound tail observed.

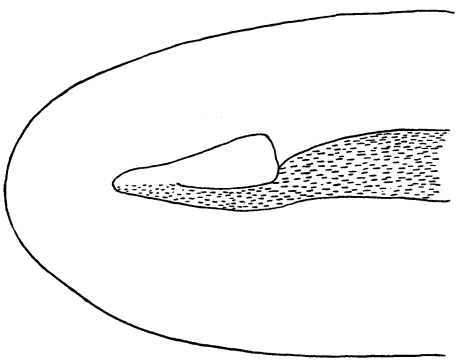


FIG. 6.

In another experiment on April 28 the tail was cut off on April 29. One of the tadpoles did not regenerate a new tail, another (R. sylvatica) had a compound tail, and one had a bifid tail, one branch being compound. Finally in another series in which nine grafts were made, one produced a compound tail, another may have contained a small amount of the major component in the new tail, six regenerated entirely like the minor component, and one was abnormal.

In addition to these cases there were three others (in the total of sixty cases) in which there was an overlapping of the two components in the tail, as in Fig. 6. In two of these the core of the new tail came from the minor component, but it is highly probable that a small addition came from the major component also. In the third case the new tail contained at its more distal end elements from both components. Unfortunately this lot was killed accidentally before they regenerated further.

In several cases double tails grew out enclosed in the same common fin, and lay usually in the same plane. In some cases the core of one of the new tails was derived from one of the components and the other from the other component. In several cases one or the other new tail received material from both components. In one of these cases it could be seen with the greatest clearness that the compound tail received material from both sources (Fig. 7). Cases of this kind are particularly convincing, since they furnish all the data for comparison between the two kinds of regenerating tissue of the two components. The dorsal tail was yellow and the upper part of the ventral tail was also yellow, and its tissue precisely like that of the dorsal tail. The pigment cells also of the yellow component extended out on to both tails.

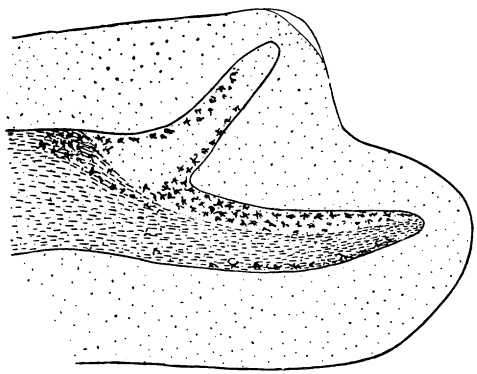


FIG. 7.

These cases of double tails are probably due to imperfect grafting, — the notochord and nerve cord of the two components not being in the same position, so that when the tail is cut off both sets of structures are exposed and a tail develops from each.

Conclusions. — The experiments demonstrate that a single tail may be formed by the regeneration of tissue derived from two species, and that in such cases there is no specific change produced in the one kind of new tissue by the other. Each kind of tissue regenerates its like, and the two kinds combine to form a single morphological organ, — the tail.

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